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WITNESS my hand this
First day of September 2003

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PROVISIONAL SPECIFICATION

Applicant:

ROYAL WOMEN'S HOSPITAL

Invention Title:

MARKER FOR EARLY STAGE CANCER SCREENING

The invention is described in the following statement:

MARKER FOR EARLY STAGE CANCER SCREENING

The present invention relates to methods of diagnosis and monitoring of cancer. In particular, the invention relates to the finding that integrin-linked kinase is overexpressed in the serum of early stage ovarian cancer patients. Hence the present invention is directed towards the development of an early screening technique for ovarian cancer.

BACKGROUND OF THE INVENTION

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

Ovarian cancer is the leading cause of death from gynaecological malignancy and the fourth leading cause of cancer death among Australian women. The cancer is highly metastatic, resulting in secondary growth to distant sites. Ovarian cancer is the fourth most common female malignancy, and is the leading cause of death from gynaecological malignancies. The majority of patients diagnosed with advanced epithelial ovarian cancer have widespread metastasis. The dismal outcome for ovarian cancer progression arises from an inability to detect the tumour at an early, curable stage. As 90% of grade I tumours can be cured by current management methods, ovarian cancer has a good prognosis for recovery if

diagnosed at an early stage. The only practicable way to identify ovarian cancer at an early curable stage is to ascertain the identity of proteins which are overexpressed in cancer cells, and hence are secreted from the cancer cells into the peritoneum, and ultimately absorbed into the circulating blood. These proteins may evoke antibody responses, suggesting that development of an antibody-based serum marker may be possible.

To date, no definite marker of ovarian cancer has been identified which is suitable for early-stage screening purpose. CA125 values are not definitive for ovarian cancer, as levels of this antigen increase in other gynaecological cancers, and sometimes even with infection. On the other hand, in certain cases of ovarian cancer no sensitivity to CA125 values has been noted, making it an unreliable marker for early screening of ovarian cancer. Hence, to prevent mortality from ovarian cancer, there is a desperate need to identify molecular markers which are detectable in blood or serum, to complement the CA125 screening test.

To prevent mortality from ovarian cancer, identification of markers which are detectable in blood or serum of cancer patients is essential to complement the use of existing tests in detecting early-stage disease. Over-expressed proteins which are secreted from cancer cells and are absorbed by circulating blood are logical candidates for development as markers using assays measuring the protein product in serum. Secreted proteins may evoke antibody responses, suggesting the development of an antibody-based serum marker.

Recent studies indicate that ovarian cancer progression depends on the combination of processes, including angiogenesis and extracellular matrix remodelling, which allows the formation of new blood vessels, followed by proliferation, invasion and migration of tumour cells. Thus a tumour which cannot sprout new blood vessels will not grow. New vessel development, and

consequently invasion and migration of tumour cells, depends upon a balance between chemical 'stimulators' or angiogenic growth factors and 'inhibitors'. A number of different angiogenic stimulators of ovarian cancer, such as VEGF, HGF, IL-6, IL-8, LPA, etc, have so far been described (Xu et al, 1998; Sowter et al 1999). However, the fundamental role of these factors in the regulation of a class of cell-surface adhesion proteins, such as integrins and their associated proteins, which mediate angiogenesis by directing the motility and migration of not only endothelial cells but also migration and invasion of tumour cells, is still unknown.

It is well known that the extracellular matrix (ECM) plays an important role in regulating cell behaviour. Both endothelial and cancer cells have the capacity to sense, and in turn respond to, the continuum of changes which occur in the composition and structure of the surrounding ECM during angiogenesis, as well as during cancer progression. Cell-specific recognition of individual matrix components is established by integrin receptors, which can then modulate cell behaviour by transmitting biochemical signals from the ECM to the cell interior (Juliano et al, 1993). The cytoplasmic tail of integrin subunits can interact with specific components of cytoskeleton (Sieg et al, 2000) or other cytoplasmic protein (Short et al, 2000). These distinct protein-protein interactions may potentiate a number of signalling events which can regulate distinct gene expression modulating cellular behaviour.

Integrin-proximal events involved in the initiation of integrin-mediated signal transduction are still poorly understood. Cell adhesion to the ECM is mediated by integrins, a family of heterodimeric transmembrane proteins comprising over 15 α and 8 β subunits which can heterodimerize in 22 different combinations (Hynes, 1992). Of the wide spectrum of integrin subunit combinations expressed on the cell

surface, αvβ3 has been identified to play an important role in angiogenesis (Brooks et al, 1994). Some invasive tumours, such as metastatic melanoma and late stage glioblastoma, also express αvβ3, which contributes to the malignant phenotype of the tumour (Petitclerc et al,1999); Gladson, 1996). Recent studies have shown high expression of αvβ3 integrin in ovarian tumours of high malignant potential (Nip et al 1995). We have also found that αvβ6 integrin is expressed in ovarian tumours, and is significantly upregulated during tumour progression (Ahmed et al, 2002), and that the invasive phenotype of ovarian cancer cells can be inhibited by ligation with monoclonal antibody against αvβ6 integrin (10D5) (Ahmed et al, 2002). On the other hand, endothelial cells exposed to angiogenic growth factors or those undergoing angiogenesis in tumours, wound, or inflammatory tissue express high levels of αvβ3 (Brooks, 1996). Disruption of αvβ3 ligation with antibody (LM609) or peptide antagonists disrupts blood vessel formation in the chick chorioallantoic membrane (CAM), quail embryo, mouse retina, rabbit cornea or arthritic knee (Eliceiri et al 2000). Histological examination revealed that these antagonists perturb the growth and maturation of new blood vessels without influencing the preexisting blood vessels (Eliceiri, B. P. et al (2000) *Cancer J Sci Am* 6(Suppl 3), S245-9). Recently two different pathways of angiogenesis have been distinguished on the basis of their dependence on the related but distinct integrins αvβ3 and αvβ5 (Friedlander, M. et al (1995) *Science* 270(5241), 1500-2). In both the rabbit corneal eye pocket and the chick chorioallantoic membrane angiogenesis assays, anti-αvβ3 mAb blocked bFGF-induced angiogenesis, whereas anti-αvβ5 antagonists blocked VEGF-induced angiogenesis. These results indicate that distinct αv integrins can not only modulate blood vessel formation, but can also regulate the invasive phenotype of cancer cells.

Recently, a novel integrin-linked kinase (ILK), a

serine-threonine kinase has been demonstrated to associate with the cytoplasmic domains of integrins β1 and β3 (Dedhar et al, 1999). ILK has been implicated in cellular control of cell-extracellular matrix interactions and cell proliferation (Dedhar, 2000), and regulates integrin-mediated cell adhesion, E-cadherin expression and extracellular matrix assembly (Wu et al, 1998). Moreover, overexpression of ILK in rat epithelial cells induces anchorage-independent cell growth in culture and tumour formation (Radeva et al, 1997). Biochemical and cell biological analyses have shown that ILK is intimately involved in the cell adhesion-dependent cell cycle progression by regulating the level and/or activity of several key components of cell cycle machinery, including cyclin A, cyclin D1 and cyclin-dependent kinases (Radeva et al, 1997).

ILK is a functional protein kinase capable of phosphorylating the cytoplasmic domain of integrin β1 subunit (Mulrooney et al, 2000), Ser473 of protein kinase B (PKB/AKT), and glycogen synthase kinase 3 (GSK3) (Persad et al, 2001); D'Amico et al, 2000). ILK has been shown to localize to focal adhesion plaques, where it localises with β1 integrin (Mulrooney et al, 2000). Evaluation of the effects of cell adhesion and growth factor stimulation has shown that the kinase activity of ILK is under stringent control by these stimuli. ILK activity is rapidly stimulated by plating on fibronectin, peaking at between 30-45 minutes and then declining rapidly to basal levels at 60 minutes. Similarly, ILK activity is stimulated rapidly by insulin and platelet-derived growth factor in serum-starved cells, peaking at 10 minutes of cell exposure (Delcommenne et al, 1998).

Both fibronectin and insulin-dependent stimulation of ILK activity are dependent on the activity of PI-3 kinase (Delcommenne et al, 1998). On the other hand, ILK induces an invasive phenotype via AP-1 transcription factor-dependent upregulation of matrix

metalloproteinases 9 (MMP-9) in brain tumour cells (Troussard et al, 2000). Recently, it has been shown that autocrine TGF-β regulates the expression of a new isoform of ILK, ILK2, which correlates with metastatic phenotype (Janji et al, 2000), indicating that in normal cells ECM and growth factor-stimulated ILK might be different in their regulation of the metastatic phenotype of cancer cells.

ILK is widely expressed in a broad range of human tissues and cells of all lineages, including freshly-isolated monocytes and peripheral blood lymphocytes (Hannigan and Dedhar 1997). ILK expression is also stimulated by the protooncogene erbB-2 in hyperplastic epidermis (Xie et al, 1998), and its expression is elevated in Ewing's sarcoma, primitive neuroectodermal tumour, medulloblastoma and neuroblastoma (Chung et al, 1998). Recently, the level of ILK expression has been shown to increase with the progression of prostate tumour grade (Graff et al, 2001). Whether dysregulated ILK expression or activity could contribute to oncogenic transformation is still not clear, but phenotypic alterations associated with the overexpression of ILK in epithelial cells indicate that it is a hallmark of oncogenic transformation (Hannigan et al, 1996).

The gene encoding PI-3 kinase is frequently activated in ovarian cancer (Shayesteh et al, 1999). As ILK is regulated by PI-3 kinase, it is possible that ILK activity may be similarly elevated in association with ovarian cancer. ILK is a novel and evolutionarily conserved serine/threonine kinase. Despite much rapid progress in the field, the physiological relevance of ILK, the role of ILK dysfunction in tumour progression, and the mechanism of action of ILK in normal and tumour cells remain to be determined.

We have now found that the immunoreactive soluble form of ILK can be detected in the serum of early stage ovarian cancer patients. Thus ILK is a candidate for

development as a biomarker, using assays measuring the protein product in serum, such as ELISA or radio-immunoassay. Moreover, our finding that ILK expression decreases after chemotherapeutic treatment and its expression correlates with the widely used ovarian cancer marker, CA125, makes it an ideal candidate to complement the widely-used CA125 marker.

SUMMARY OF THE INVENTION

In a first aspect, the invention provides a method of detection of ovarian cancer, comprising the step of determining the level of ILK in a sample of a biological fluid from a subject suspected to be suffering from ovarian cancer, wherein the presence of ILK is an indication of the presence of the cancer.

In a second aspect, the invention provides a method of monitoring the efficacy of treatment of ovarian cancer, comprising the step of determining the level of ILK in a sample of a biological fluid from a subject suspected to be suffering from ovarian cancer, wherein a decrease in ILK levels is an index of efficacy of the treatment.

In a third aspect, the invention provides a method of detection of recurrence of ovarian cancer, comprising the step of determining the level of ILK in a sample of a biological fluid from a subject undergoing treatment for ovarian cancer, wherein the presence of detectable ILK is an indication of recurrence of the cancer.

In a fourth aspect, the invention provides a method of assessing the severity of ovarian cancer, comprising the step of quantitatively determining the level of ILK in a biological fluid of a subject diagnosed with, or suspected to be suffering from, ovarian cancer, and optionally correlating the levels of ILK with one or more other markers of ovarian cancer. Preferably the

other marker is CA125.

In all four aspects of the invention the biological fluid may be blood, plasma, or serum. The person skilled in the art will readily be able to determine whether other biological fluids, such as saliva or urine, could also be used; however, it will be appreciated that in these cases more sensitive detection methods may be required.

Preferably the biological fluid is serum.

Optionally the methods of the invention also comprise the step of determining levels of another ovarian cancer marker, such as CA125, in the biological fluid, using conventional methods.

The levels of ILK may be determined by any convenient method, including but not limited to ELISA, radioimmunoassay, chemiluminescence, real time polymerase chain reaction, and the like. Specific antibodies directed against ILK are commercially available, and may be used in such methods.

The mammal may be a human, or may be a domestic or companion animal. While it is particularly contemplated that the compounds of the invention are suitable for use in medical treatment of humans, they are also applicable to veterinary treatment, including treatment of companion animals such as dogs and cats, and domestic animals such as horses, cattle and sheep, or zoo animals such as non-human primates, felids, canids, bovids, and ungulates.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the results of immunohistochemistry using a polyclonal antibody against ILK. No immunoreactive ILK

was present in normal ovaries (A), but the expression of ILK in two grade III ovarian tumour tissues (serous, panel B and endometrioid, panel C) was detected.

Figures 2A and 2B show ILK expression in the serum of three normal, three borderline, three grade I and three grade III cancer patients, as detected by the semi-quantitative Western blotting technique using a polyclonal antibody against ILK.

Figure 2A:ILK expression in serum of normal adults (N) and ovarian cancer patients (borderline, B/L, grade 1; GI and grade III, G3).

Figure 2B:ILK expression in ascitic fluid (AS) from ovarian cancer patients.

Figures 2C and 2D summarize the findings of densitometric analysis of Figure 2A, demonstrating the enhancement of ILK expression in the serum of grade I ovarian cancer patients. This indicates that quantification of ILK in the serum of ovarian cancer patients, for example by ELISA or radio-immunoassay, can be used as a diagnostic marker for screening purposes.

Figure 3 shows the results of immunohistochemical analysis of normal ovarian epithelium and a grade III serous ovarian tumour, using the same antibody as in Figure 1.

Figure 3A shows the results of Western blot analysis of expression of ILK in a normal ovarian cell line (HOSE) and ovarian cancer cell lines, and Figure 3B demonstrates the upregulation of ILK expression by treatment with ascitic fluid (10%) for 24 hours.

Figures 4A and 4B compare expression of ILK (Panel A)and of the ovarian cancer marker CA125 (Panel B) in the serum of ovarian cancer patients, before and after six cycles of carboplatin and paclitaxel chemotherapy. The filled bars in both figures represent values before treatment, while the open bars are values after treatment.

Figure 5 shows a schematic diagram illustrating a model of ILK regulation and its contribution to associated phenotypic changes in ovarian cell lines.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described in detail by way of reference only to the following non-limiting examples and drawings.

We propose that similar extracellular interactions and intracellular signal transduction pathways characterise the processes of endothelial cell angiogenesis and ovarian epithelial cell metastasis. In this study, we characterise the role of ILK in these processes.

The specific aims of this study were:

(a) To quantify changes in ILK activity and expression associated with the onset and progression of ovarian cancer;

(b) To quantify ILK activity and expression in normal and cancerous ovarian cell lines;

(c) To establish the effects of ECM molecules (eg fibronectin, collagen IV), angiogenic growth factors (eg VEGF, HGF, LPA) and ascitic fluid on ILK activity and expression, and metastatic phenotype (eg ECM degradation, cell migration);

(d) To identify the signalling pathways involved in the above mentioned induction of ILK expression and metastatic phenotype; and

(e) To establish the role of ILK in the induction of a metastatic phenotype by the use of specific inhibitors of ILK activity (KP-SD-1 and KP-SD-2).

The data presented below show that:

1. Immunoreactive ILK (irILK) is overexpressed in ovarian tumours and in serum collected from patients with early stage ovarian cancer;

2. In ovarian cancer tissues, irILK is present in the endothelial cells lining the new blood vessels.

3. irILK is overexpressed in ovarian cancer cell lines (eg OVHS 1, HEY, PEO.36, OAW 42 and SKOV 3), but not in normal ovarian cell lines (eg HOSE);

4. Overexpression of irILK can be induced by incubation with ascitic fluid in ovarian tumour cell lines but not in normal ovarian cell lines; and

5. irILK is also present in ascitic fluid from patients with ovarian cancer.

These data are consistent with the hypothesis that overexpression of ILK is an early event in the onset of ovarian cancer. Thus overexpression of ILK may represent a novel and efficient mechanism for enhancing angiogenesis, which is a pre-requisite for tumour cell growth and metastasis.

Example 1 Immunohistochemical analysis

Figure 1 shows an immunohistochemical comparison between samples of normal ovarian epithelium and of grade III serous and endometrioid ovarian tumours. Tissue sections were stained with primary ILK antibody (Zymed, USA) followed by amplification with biotin-streptavidin horse radish peroxidase. The complex was visualised using diaminobenzidine (DAKO, Denmark). No expression of ILK was observed in normal ovarian epithelium, as shown in Figure 1A. In grade III serous and endometrioid ovarian tumours, shown in Figures 1B and 1C, high expression of ILK was detected in blood vessels lining the epithelium, but scattered epithelium staining was also observed.

The lack of expression of ILK in normal epithelium and expression of ILK in advanced stage tumours indicates that ILK is critical for ovarian cancer progression. The strong expression of ILK in endothelial cells and in blood vessels of grade III tumour indicates that ILK may be involved with angiogenesis.

However, the most interesting observation was the increment in ILK expression in ovarian tumour tissues as the cancer progresses. To our knowledge the presence of ILK in serum and ascites fluid of cancer patients has not been previously reported. Although it is known that ILK

expression in tissues is up-regulated in conditions such as cancer, diabetes, and proteinuria, a secretory form of ILK has not been shown previously.

Example 2 Expression of ILK in normal and ovarian cancer patient's serum and ascites

A range of normal and ovarian cancer tissues and serum was analysed for the expression of ILK. ILK expression was also observed in ascitic fluid of ovarian cancer patients. ILK expression was detected in tissues by immunohistochemistry, serum, and ascitic fluid by Western blotting under non-reducing conditions, using a polyclonal antibody against ILK.

Whole blood (2ml) was collected by venepuncture into plain collection tubes, and allowed to clot at room temperature for 30 min. Samples were then centrifuged at 2000g for 10min after which serum was collected. An aliquot (100µl) was removed for the determination of total protein. Serum was stored at -80°C until analysed.

Blood specimens were thawed at room temperature and incubated with 5 volumes of Laemli buffer. Specimens containing equal amounts of protein (60 µg) were electrophoresed on a 10% SDS-PAGE gels under non-reducing conditions, and then transferred to nitrocellulose membranes. Membranes were probed with primary ILK antibody (Upstate Biotechnology, USA) followed by peroxidase-labelled secondary antibody (Amersham, UK) and visualised by the ECL (Amersham, UK) detection system according to the manufacturer's instructions.

Figure 2A shows ILK expression in serum of normal adults and ovarian cancer patients. Figure 2B shows ILK expression in ascitic fluid from ovarian cancer patients.

Densitometric measurement was used to estimate the level of ILK expression in the serum of three normal, three borderline, three grade I and three grade III cancer patients, as detected by the semi-quantitative Western

blotting technique illustrated in Figure 2A. These results are summarised in Figures 2C and D. This indicates that quantification of ILK expression, for example by ELISA or radio-immunoassay, in the serum of ovarian cancer patients can be used as a diagnostic marker for screening purposes.

Example 3 Expression of ILK in ovarian cancer cell lines

The pattern of expression of ILK in ovarian cancer cell lines was analysed. All ovarian cancer cell lines express ILK. As ascitic fluid from ovarian cancer patients is a rich source of ECM molecules such as fibronectin and angiogenic growth factors such as VEGF, IL-6, IL-8, LPA, HGF, TNF-α, we investigated the possibility of alteration in ILK expression in response to ascitic fluid in normal ovarian and cancer cells.

Cells from a normal ovarian cell line (HOSE) and ovarian cancer cell lines were treated with ascitic fluid (10%) for 24 hours. The ascitic fluid from five different patients was used in five separate experiments. Cells were harvested with trypsin-versene, lysed by sonication with three 30 s pulses, and centrifuged at 900 g for 20 min. The level of expression of ILK was determined in the cell lysates using a polyclonal antibody specific against ILK. Figure 3 shows results from one representative experiment.

As shown in Figure 3B, the expression of ILK was enhanced in response to ascitic fluid in ovarian cancer cells, while no such effect was observed in normal HOSE cells, shown in Figure 3A. We are carrying out experiments to determine whether these changes result in phenotypic changes in normal ovarian cells.

Example 4 Effect of anti-cancer chemotherapy on ILK expression

The effect of anti-cancer chemotherapy on levels of ILK expression in serum was assessed, using levels of the widely-used ovarian cancer marker CA 125 as a comparison.

Figures 4A and 5B compare levels of ILK and of CA125 in the serum of ovarian cancer patients, before and after six cycles of carboplatin and paclitaxel chemotherapy. The filled bars in both figures represent values before treatment while the open bars are values after treatment. The results show that the expression of ILK correlates with CA125 values. However, in cases of drug resistance or recurrence, where the CA125 value is above 35U/ml (the normal limit), ILK expression remains relatively unchanged (third bars in both figures).

DISCUSSION

Our preliminary findings suggest that ILK couples with ECM molecules and growth factors to downstream signalling kinases regulating ovarian cancer cell survival and growth. Without wishing to be bound by any proposed mechanism, we have formulated a model of ILK regulation and its contribution to associated phenotypic changes in ovarian cell lines on the basis of our preliminary findings and review of the present literature, and this is shown in Figure 5. According to this model, integrin-linked kinase interacts with growth factor receptors and ECM to regulate downstream signaling molecules that modulate cell survival, initiation of G1/S phase of cell cycle, invasion and metastasis of endothelial and cancer cells. ILK activity is regulated in a phosphoinositide 3-kinase (P-I3 kinase)-dependent manner, but the involvement of the MAP kinase pathway, which is regulated by

integrins, is not yet defined. Activated ILK phosphorylates PKB/Akt, resulting in its activation. Activated ILK can also phosphorylate and inhibit the activity of glycogen synthase kinase (GSK-3), which in turn results in the activation of β-catenin and transcription factors such as AP-1, NF-κB, Lef-1, etc. ILK also regulates the expression of cyclin D1, via its effect on GSK-3. The activation of PKB/Akt and inhibition of GSK-3 by ILKs induce suppression of apoptosis and promotion of cell survival, leading in the case of cancer cells to migration, invasion and metastasis.

The loss of cell-cell adhesion induced by overexpression of ILK in rat intestinal and mammary epithelial cells, due to a dramatic decrease in E-Cadherin expression, is accompanied by translocation of β-catenin to the nucleus (Somasiri et al, 2001). ILK overexpression leads to dramatic relocalisation of β-catenin to the nucleus, although the level of free cytosolic β-catenin does not seem to increase (Novak et al, 1998). This is probably a result of the unusual elevation of Lef1, the transcription factor partner of β-catenin. The gene encoding E-cadherin can be negatively regulated by Lef1-β-catenin complex, thus providing a potential mechanism of ILK-induced loss of cell adhesion in intestinal and mammary carcinomas. Another consequence of ILK overexpression in epithelial cells is epithelial-mesenchymal transition, which includes enhanced fibronectin assembly, loss of expression of keratins and enhanced expression of vimentin (Somasiri et al, 2001). On the other hand, inhibition of ILK by synthetic inhibitors in colon cancer cells suppresses β-catenin Lef1 dependent transcription, and causes upregulation of E-cadherin (Tan et al, 2001), indicating that ILK expression is critical for maintaining cell-cell adhesion and epithelial conformation.

Both stable and transient transfection of ILK in epithelial cells results in the phosphorylation of GSK3 on

Ser9 (D'Amico et al, 2000). This is possibly mediated through the activation of PKB/AKT by phosphorylation at Serine473. GSK3 phosphorylates cyclin D1 on Thr286. These actions of ILK are regulated by PI-3 kinase, indicating that ILK is a receptor-proximal effector of PI-3 kinase signals in epithelial cells (Delcommenne et al, 1998). ILK overexpression, on the other hand, can enhance the expression of cyclin D1 and activation of cyclin E-associated kinases, resulting in hyperphosphorylation of retinoblastoma protein, and expression of p21 and p27 cyclin-dependent kinase inhibitor with reduced inhibitory activity (Radeva et al, 1997). These results suggest that overexpression of ILK induces signalling pathways, resulting in the stimulation of the G1/S phase of cell cycle which under normal conditions is regulated by integrin engagement. Thus overexpression of ILK in epithelial cells may override adhesion-dependent regulation of cellular function.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

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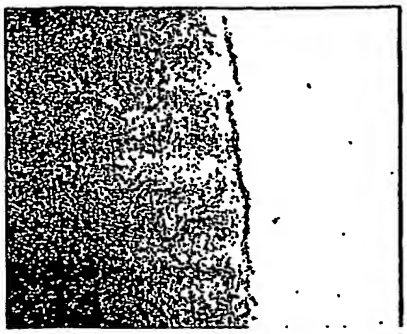


Figure 1a

Figure 1b

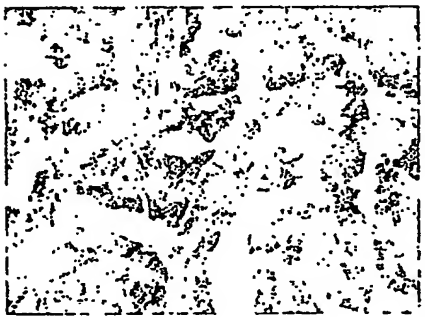


Figure 1c

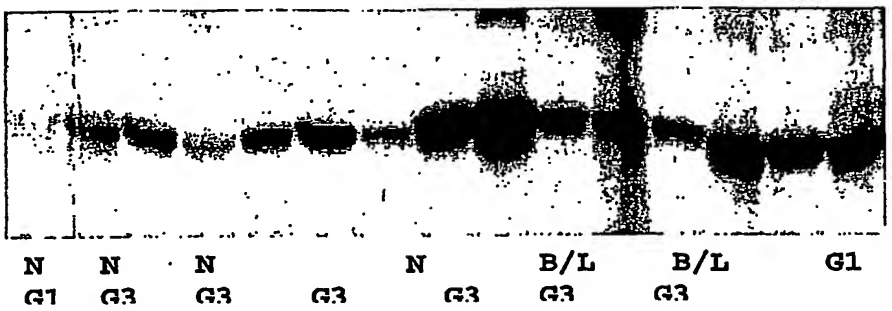
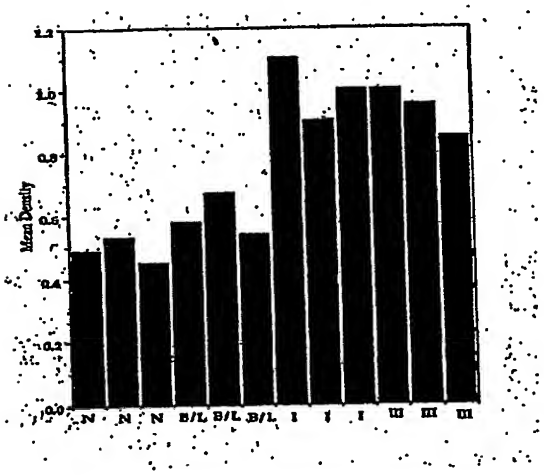


Figure 2A

ILK 59 kDa

ILK-Std Asitic fluid

Figure 2B



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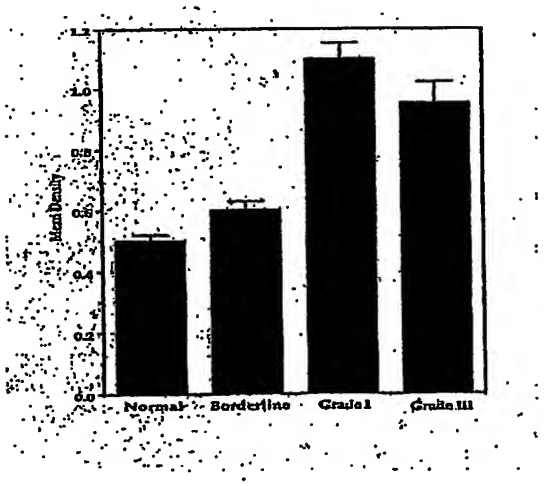

Figure 2d

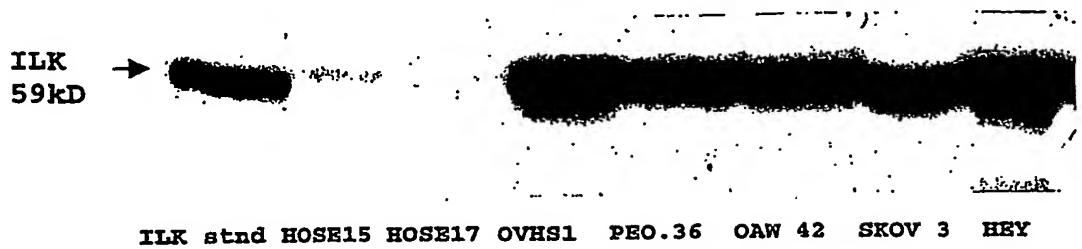

Figure 3A

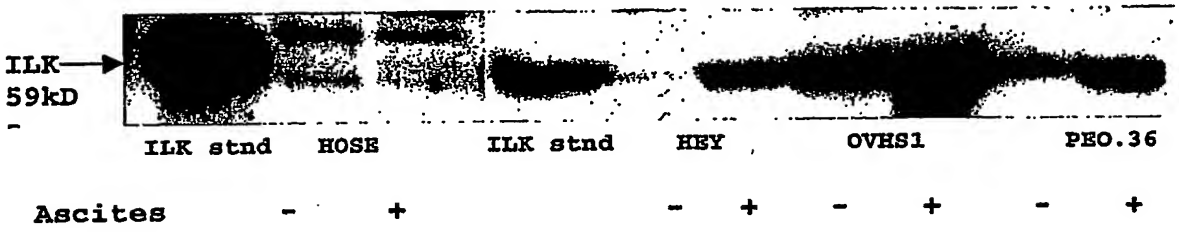

Figure 3B

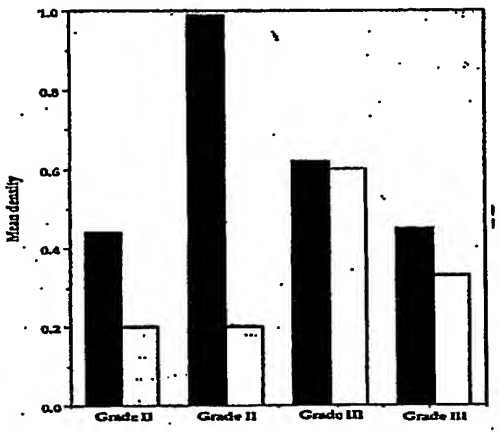

Figure 4A

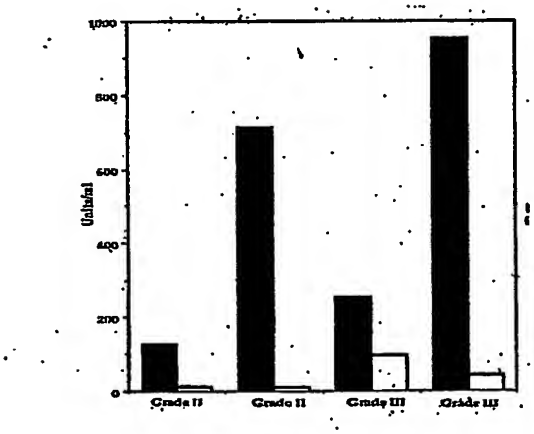

Figure 4B

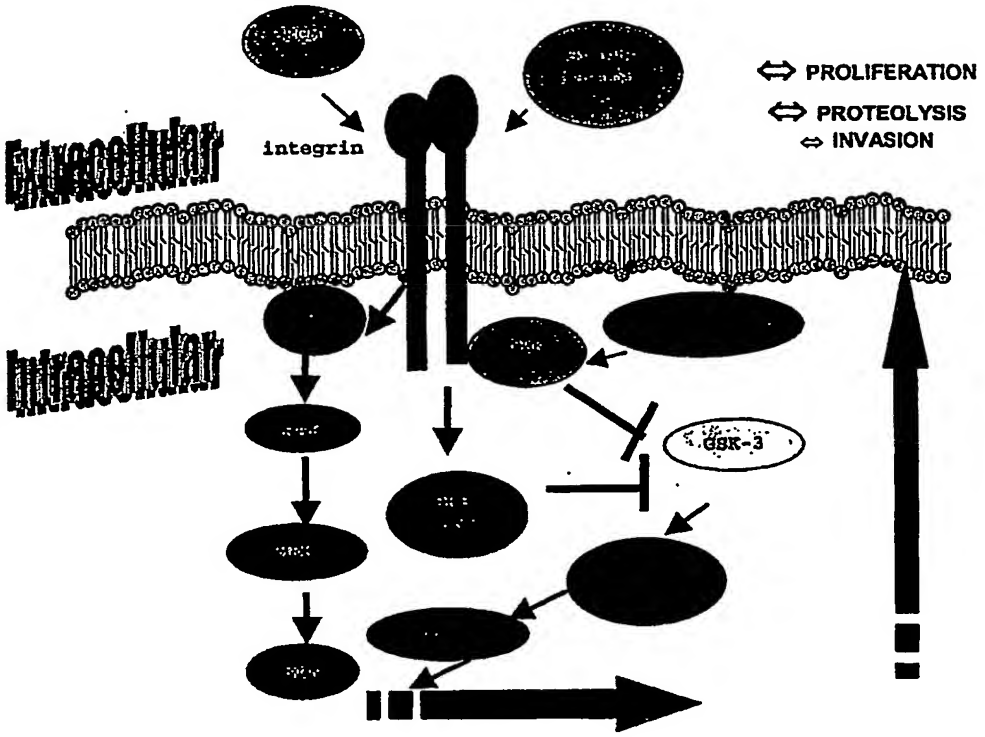
integrin
⇔ PROLIFERATION
⇔ PROTEOLYSIS
⇔ INVASION
GSK-3
Protein
Expression
e.g. MMP-9,
serine
proteases?,

Figure 5